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High root temperature affects the tolerance to high light intensity in *Spathiphyllum* plants

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ABSTRACT

Spathiphyllum wallisii plants were sensitive to temperature stress under high illumination, although the susceptibility of leaves to stress may be modified by root temperature. Leaves showed higher tolerance to high illumination, in both cold and heat conditions, when the roots were cooled, probably because the chloroplast were protected by excess excitation energy dissipation mechanisms such as cyclic electron transport. When the roots were cooled both the activity of electron donation by NADPH and ferredoxin to plastoquinone and the amount of PGR5 polypeptide, an essential component of cyclic electron flow around PSI, increased. However, when the stems were heated or cooled under high illumination, but the roots were heated, the quantum yield of PSII decreased considerably and neither the electron donation activity by NADPH and ferredoxin to plastoquinone nor the amount of PGR5 polypeptide increased. In such conditions, the cyclic electron flow cannot be enhanced by high light and PSII is damaged as a result of insufficient dissipation of excess light energy. Additionally, the damage to PSII induced the increase in both chlororespiratory enzymes, NDH complex and PTOX.

1. Introduction

Plants are frequently exposed to environmental stress, both in natural and agricultural conditions. It is also common for plants to suffer more than one abiotic stress at a given time, for example, high illumination and high or low temperatures, which negatively affect the photosynthetic process [1,2]. When leaves are exposed to more light than they can use for photosynthesis, the excess of absorbed light energy may lead to the production of toxic species and damage the photosystems. PSII is the most sensitive site to photoinhibition, whereas PSI is more stable; although when high illumination accompanies low temperatures, PSI could be photoinhibited [3]. Recently, it has been reported that root temperature is a very important factor for the response and susceptibility of leaves to high light and chilling stress [4,5]. For example, visible damage to *Oryza sativa* leaves exposed to high light was observed when only leaves, but not roots, were chilled, whereas no visible damage was observed when both leaves and roots were chilled simultaneously [4]. Additionally, when only leaves, but not roots, were chilled the PSII was photoinibited, but not PSI [4]. These authors suggested that leaf chilling accompanied by high root temperature blocks the electron transport between Q_A and Q_B in PSII, leading to the over-reduction of PSII in the light, although the biochemical basis for this behavior has not been established [5].

Photosynthesis in chloroplasts involves vectorial electron transfer from water in the lumen to $NADP^+$ in the stroma by carriers. Besides this major pathway, alternative electron transfer pathways, involving non-photochemical reduction or the oxidation of plastoquinones at the expense of stromal electron donors or acceptors, have been proposed based on functional measurements. These additional reactions cover two main concepts, one based on the cycling of electrons around PSI [6–8] and the other on chlororespiration [9,10], which consists of electron transfer reactions from stromal reductants to O_2 through the plastoquinone pool [7,11,12]. Two thylakoidal enzymes, both of which are important in chlororespiration, have been molecularly characterized: the plastid-encoded NADH dehydrogenase (NDH) complex [13,14] and the nucleus-encoded plastid-localised terminal oxidase (PTOX) [15–17]. The NDH complex is an entry point for electrons into the photosynthetic electron-transport chain, involving the non-photochemical reduction of plastoquinones, while PTOX is a point

Abbreviations: F, fluorescence yield; F_m, maximal fluorescence yield in the dark-adapted state; F_0, minimal fluorescence yield in the dark adapted state; F'_m, maximal fluorescence yield in the light adapted state; F_v, variable fluorescence; NDH, NADH dehydrogenase; Nq, non-photochemical quenching; PAM, pulse amplitude modulation; PPFD, photosynthetic photon flux density; PS, photosystem; PTOX, plastid terminal oxidase; Y(II), effective PSII quantum yield.

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of electron transfer from plastoquinol to molecular oxygen, resulting in the formation of water in the stroma and reducing the formation of reactive oxygen species [11]. In addition to chlororespiration, the NDH complex is involved in the cyclic electron flow around PSI [18]. Two parallel cyclic pathways exist around PSI [18], one involving the NDH complex and the other sensitive to antimycin A, in which the thylakoid membrane protein encoded by pgr5 gene (PGR5) is an essential component [19–21]. The physiological role of the chloroplast electron pathways operating around PSI is difficult to establish. Although these reactions probably do not play a major role during photosynthesis under optimal conditions [22–25], they probably participate in the flexibility of the electron transfer reactions required to balance ATP/NADPH requirements when photosynthesis operates under changing environmental conditions [12,26–31]. Several studies have proposed that chlororespiratory components may be involved in the protective or adaptive mechanisms of plants in response to environmental stress, such as heat, water deficit and high light [15,32–45]. However, the possible role of chlororespiratory components in conditions of cold stress remain unclear [12]. It was reported that PTOX levels increased in alpine plant species acclimated to high light and low temperature [39,46] and in cold-acclimated *Arabidopsis thaliana* plants [47], although no increase in the NDH complex under cold stress was observed [47].

The present work studies the effects of heat and cold in the root and stem, as well as the involvement of chlororespiratory enzymes and PGR5, on the tolerance of photosynthesis to high light intensity in *Spathiphyllum wallisii*, a plant of tropical origin with growth temperatures ranging between 15 °C and 25 °C.

2. Materials and methods

2.1. Plant material and growth conditions

S. wallisii plants were grown in 500 mL pots at 22–25 °C in the greenhouse with a natural photoperiod (irradiation maxima of around 200 μmol m^{-2} s^{-1} PPFD) and watered to avoid drought stress (control conditions). To simulate stress conditions, adult plants were transferred to cultivation chambers, where they were exposed to one 18 h photoperiod of high light intensity (1060 μmol m^{-2} s^{-1} PPFD, supplied by a white light lamp of 100 W Flood OSRAM, Augsburg, Germany) and different temperature treatments. The photoperiod was followed by a 6 h night-period. The temperature treatments were as follows: 24 °C/24 °C; 10 °C/10 °C; 37 °C/10 °C, 37 °C/37 °C and 10 °C/37 °C, representing stem/root temperature, respectively. To change the temperature of the root with respect to the stem, a thermostatic circulator (LKB, GmbH, Germany) with a coil around the pot was used. In each treatment, the photoperiod temperature was the same as during the night-period.

2.2. Isolation of thylakoid membranes

Chloroplasts were isolated from leaves after the night period, as described by Quiles and Cuello [13], using an extraction buffer (pH 7.6) containing 0.35 M sucrose, 25 mM Na-Hepes, 2 mM Na_2-EDTA, 2 mM ascorbic acid, 4 mM dithiothreitol, 10 mM $MgCl_2$ and 1 mM phenylmethylsulfonyl fluoride. As reported previously, a comparison of cytochrome *c* oxidase-specific activity and polypeptide profiles in mitochondrial and chloroplast fractions indicated that the chloroplast preparation was essentially mitochondria-free [48]. The chloroplasts were washed twice and osmotically broken with 10 mM Tricine, 10 mM NaCl and 10 mM $MgCl_2$ (pH 7.8) buffer, as described previously [40]. The thylakoid membrane pellet was resuspended in buffer (pH 7.5) containing 200 mM sorbitol, 130 mM KCl and 5 mM potassium phosphate at a chlorophyll concentration of 0.4 mg mL^{-1}, thus providing the suspension of the thylakoid membranes.

2.3. Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured in the thylakoid membrane suspension (50 μg Chl mL^{-1}) using a PAM-210 chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) and chlorophyll fluorescence was imaged, using the MINI-version of the Imaging-PAM (Heinz Walz GmbH, Effeltrich, Germany), in selected leaves attached to plants after the night-period. The fluorometer used employs the same blue LEDs for the pulse modulated measuring light, continuous actinic illumination and saturation pulses. The minimal fluorescence yield (F_0) and the maximal fluorescence yield (F_m) were measured in dark-adapted samples. F_0 was measured at a low frequency of pulse modulated measuring light, while F_m was measured with the help of a saturation pulse. Selected leaves were illuminated for 2 min with actinic light (55 μmol m^{-2} s^{-1} PPFD), measuring the fluorescence yield (F) and the maximal fluorescence yield in illuminated samples (F'_m). The maximal quantum yield of PSII ($F_v/F_m = (F_m - F_0)/F_m$), the effective PSII quantum yield of illuminated samples ($Y(II) = (F'_m - F)/F'_m$), photochemical quenching (qP), non-photochemical quenching (NPQ), $1 - F'_v/F'_m$ and "excess" ($(1 - qP)F'_v/F'_m$) were automatically calculated by the ImagingWin software.

2.4. PSI activity measurements

The PSI activity was measured spectrophotometrically as described by Xiao et al. [49]. One unit (U) of photosystem activity is defined as the sample amount which oxidized 1 μmol of 2,6-dichlorophenol indophenol per minute in the reaction conditions.

2.5. Gel electrophoresis and immunoblot analysis

SDS-PAGE was carried out in a linear gradient of 10–20% polyacrylamide gel (2.5% bis-acrylamide), as previously described [13]. Protein samples were denatured by the addition of 3.5% (w/v) SDS and 5% (v/v) mercaptoethanol and heating for 10 min at 70–80 °C before being subjected to SDS-PAGE (25 μg of the thylakoid proteins per well). Prestained SDS-PAGE standards (Bio-Rad Laboratories, USA) were used for immunoblot analyses. After SDS-PAGE, the polypeptides were electroblotted from gels onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, USA). Transfer and immuno-detection of the blotted protein was carried out as described previously [13]. The secondary antibody was conjugated to alkaline phosphatase (Sigma, USA). Controls in which the primary and/or secondary antibodies were omitted during incubation did not reveal any bands.

2.6. Other determinations

Protein was quantified using the method of Lowry et al. [50] after precipitation with 10% (w/v) trichloroacetic acid. Chlorophylls were determined by Lichtenthaler and Wellburn's [51] method using 80% (v/v) acetone as solvent. Densitometric analysis and estimation of the polypeptide molecular masses were performed by ACTIB 1D digital image analyzer (Microptic, Barcelona, Spain).

3. Results

3.1. Photosynthetic parameters

The fluorescence imaging technique was used to assess photosynthesis in intact leaves from plants exposed to different